AEROSOL INFECTION OF MAN WITH *PASTEURELLA TULARENSIS*

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Tularemia acquired by natural means is manifested by a variety of clinical syndromes in the human host. If one assumes that the existing literature reflects accurately the true state of affairs in this disease, tularemia in the United States is predominantly of the ulceroglandular, oculoglandular, and glandular varieties (3–5, 9). Thus, among the several large series of human cases reported in this country, only 5 to 10% of patients were afflicted with what is generally referred to as typhoidal tularemia. When there has been obvious contact with a potentially infected arthropod or warm-blooded animal, principally of the rodent group, the appearance of an eschar and enlargement of regional lymph nodes clearly establishes the source and pathogenesis of infection. Development of pneumonia during the course of ulceroglandular tularemia may reasonably be ascribed to the circulation of *Pasteurella tularensis* in the blood. This is properly referred to as secondary or embolic pneumonia. Twelve to fifteen per cent of patients with ulceroglandular disease exhibited this manifestation of tularemia prior to the advent of effective antimicrobial drugs (1, 3, 6). By contrast, approximately 50% of patients with tularemia, in whom a portal of entry of microorganisms was not discernible, developed some pleuropulmonary manifestation of the disease (12). This obvious difference in incidence of lung involvement strongly suggests that the portal of entry may be the respiratory tract. However, it should be recognized that the mechanism of infection in so-called typhoidal tularemia is still a matter of controversy, there being those who doubt the importance or even the existence of primary pneumonic tularemia. One of the objectives of this presentation will be to marshal evidence in support of the concept that primary respiratory tularemia occurs as a naturally acquired as well as induced disease. Information obtained from the study of induced disease may lead to a better understanding of pathogenetic mechanisms which are operative in typhoidal tularemia.

The unique capacity of *P. tularensis* to adapt to various environments is reflected in the multiple epidemiological patterns observed throughout the world. For example, the maintenance of endemic foci among water rats in certain parts of the Soviet Union is associated with waterborne outbreaks of tularemia among human beings in that country (11). During World War II, hundreds of thousands of people contracted tularemia as a result of the breakdown in public health and sanitation. In the Rostov area alone, 14,000 cases were encountered in January 1942, and tularemia is alleged to have been a serious cause of disability in Russian troops. Although ulceroglandular and typhoidal tularemia are also frequently observed in the Soviet Union, the obvious relationship which exists between consumption of water contaminated with *P. tularensis* and the occurrence of anginoglandular as well as abdominal tularemia serves to delineate clearly the clinical syndromes and most likely portals of entry associated with various types of tularemia in the human host. The extreme rarity of pharyngeal or tonsillar as well as abdominal tularemia in the United States seems to be evidence enough that few people in this country acquire the disease via the alimentary tract. Conversely, it is unlikely that typhoidal tularemia is acquired in this manner in view of the paucity of patients manifesting signs of anginoglandular and abdominal disease.

Pneumonic tularemia has been recognized as a serious disease for many years. Sixty-nine of ninety-five (73%) patients who had succumbed to tularemia and who were subjected to postmortem examination presented lesions of pneumonic tularemia (12). Prior to the advent of effective antibiotic therapy, 40 to 60% of patients who developed pleuropulmonary tularemia died of this disease. Of the 268 cases of tularemic pneumonia reviewed by Stuart and Pullen (12), 64% had ulceroglandular disease and 36% were said to have had typhoidal tularemia in the absence of a visible primary lesion. Among the 115 who were aware of contact with a potential source of infection, 92 had handled rabbits, 13

had been in contact with ticks, 3 with squirrels, 2 with deerflies, and the remainder with opossums, cats, and rats.

The most frequently observed lung lesion was a lobular pneumonia which involved one or several lobes. These lesions appeared as discrete nodules and localization in the lung periphery resulted in pleural effusion. The inflammatory process led to necrosis of lung parenchyma and caseous lesions that eventually cavitated. Cellular response was said to have been primarily mononuclear with alveolar exudates of lymphocytes, red cells, and plasma cells. Interstitial reaction usually was not marked.

Roentgenographic findings vary from multilobular pneumonia to single, small circumscribed infiltrations. Hilar adenopathy may be a part of the roentgen picture as well as pleural effusion. The degree of lung involvement revealed by roentgen examination is frequently out of proportion to the paucity of clinical signs. Whether pneumonia occurs in the presence of ulceroglandular disease or as a part of typhoidal tularemia, symptoms of lung involvement usually predominate. Several days to several weeks after the appearance of an eschar and regional adenopathy, embolic pneumonia characterized by the sudden onset of increased fever, chest pain cough, and dyspnea may appear. Generally, the pulmonary symptoms are less severe than in most other forms of pneumonitis, and the laboratory worker may become febrile and complain of severe headache and malaise without any suspicion of the pneumonic process which is evolving as the illness progresses.

Pleural effusion is most frequently seen in those patients who have had pneumonic tularemia for several days or more. Cough is nonproductive in more than 50% of patients with pneumonic tularemia and rarely is sputum produced in large amounts.

In the series reported by Stuart and Pullen (12), there was a higher incidence of pneumonia among Negro patients with tularemia and a much higher mortality rate as compared, in both instances, to white patients with tularemia studied in the same hospital.

Recent experience with laboratory-acquired as well as artificially induced tularemia has led to a better understanding of the clinical syndromes associated with airborne infection of man with *P. tularensis*. In a review of 42 patients by Overholt and his colleagues (8), a laboratory source of contamination was assured and it was the opinion of the authors that respiratory exposure was the most likely mode of infection. Thirty-nine of these patients experienced a tularemic infection which was not associated with a visible primary lesion or evidence of alimentary infection of the varieties described by Soviet workers. One-half of these patients were aware of a conspicuous break in technique, either due to personal error or failure of equipment. On the basis of these histories, the estimated incubation period in 17 was 3 to 6 days; in 4 it was somewhat longer, 7 to 12 days. Roentgenographic evidence of pneumonitis was observed in approximately one-half of those with typhoidal tularemia. The clinical syndrome in this group differed from those without X-ray changes only in the roentgenologic features and the presence in some of pleural pain and other evidence of pneumonitis.

It is of more than passing interest that all of these patients had received multiple inoculations of nonviable vaccine, either phenolized or acetone extracted. Agglutinating antibody titers ranged from 1:10 to 1:320 prior to the onset of symptoms and most of the patients experienced a rise in bacterial agglutinins during the course of their infection. In connection with the status of their immunity to tularemia, it is pertinent to observe that only six of this group had illnesses which were classified as severe. As will be noted subsequently, this is in sharp contrast to the reaction of presumably nonimmune volunteers exposed to minimally infective doses of *P. tularensis*.

The clinical syndrome observed in these laboratory workers consisted of fever, chilliness, headache, sweating, malaise, myalgia, backache, and anorexia. Soon after the onset of these symptoms, most patients noted sore throat, coryza, cough, and vague substernal pain. Severity of illness was not related to the presence of pneumonitis although chest pain and cough were more obvious in patients with pneumonitis.

P. tularensis was isolated from sputum, pharyngeal, and gastric washings with relative ease when the use of guinea pigs and artificial media were combined. Although 85% of sputum specimens tested yielded the organism, adequate samples of sputum were obtained from only 12 patients and 11 of these also had positive pharyngeal or gastric washings. Gastric washings yielded

the causative organism in 80% of patients including those with and without pneumonitis. Pharyngeal washings were positive in 67%. *P. tularensis* was recovered from the blood only once; this was in the case of a severely ill man with widespread lung involvement.

As many of these patients were thought to have been infected with a streptomycin-resistant variant of *P. tularensis*, broad-spectrum antibiotic therapy was employed where specific treatment was deemed essential or advisable. The most commonly employed regimen consisted of 2 g of tetracycline daily for a period of 7 to 10 days; this was followed by clinical evidence of relapse in only 5 of 17 patients so treated.

Overholt has emphasized the difficulty of assessing the effect of vaccine on the course of disease experienced by laboratory workers. In the absence of any method for detecting exposure to virulent organisms, it is virtually impossible to ascertain the exposure rate among these people. Furthermore, nothing is known of the degree of the exposure. Therefore, any attempt to assess the effect of artificially acquired immunity on the incidence of disease must certainly also take into account the degree of illness experienced by previously vaccinated personnel. It appears from these data that an unusually high incidence of mild infection was encountered in this group, only 6 out of 42 having severe disease. On the basis of our experience in subsequent studies, it seems logical to conclude that this attenuation of infection may be properly ascribed to the effect of vaccine.

During the course of our recent studies designed specifically to assess the efficacy of vaccines in the prevention of aerogenic tularemia, we had opportunity to define the reaction of immunized and nonimmune human beings to this kind of challenge. Volunteers previously vaccinated with a living, attenuated strain of *P. tularensis* (2) or with the chemically fractionated cell-wall antigen of Larson (10) were exposed to a virulent respiratory challenge varying in size from 10 to 1,000 human infectious doses (200 to 20,000 organisms).

Among the control subjects, clinically overt disease appeared within 3 to 5 days following exposure to a small-particle aerosol of a few seconds' age. Only the incubation period was affected by the size of the inoculum. All controls developed full-blown disease characterized by the abrupt onset of fever, headache, chilliness, sore throat accompanied by malaise, marked myalgia, and backache. Within 24 hr, patients refused food and complained of nausea. There was frequently substernal pain of an oppressive type; few had sharp pleural pain aggravated by breathing. Most patients had coryza; cough, although uniformly present, was nonproductive. Physical examination failed to reveal little more than pharyngeal injection and the general appearance of moderately severe illness. Fevers ranged between 103 and 104 F within the first 18 to 24 hr of illness. Some depression of total leukocytes with a predominance of lymphocytes was observed frequently during the acute phase of the illness. During this period, there was also an increase in erythrocyte sedimentation rate and the appearance of C-reactive protein. The latter abnormality was particularly useful as a sensitive indicator of microbial activity, since it disappeared as the illness remitted and reappeared promptly with febrile relapse.

Roentgenographic evidence of pneumonitis appeared in 8 of 32 volunteers exposed to respiratory challenge. Two of these roentgenologic abnormalities appeared among the eight controls. All but one of the eight had a small discrete pulmonary lesion. The one exception, recipient of a live vaccine, had a more diffuse bronchopneumonia.

Vaccinees were classified into three groups: (i) those who remained asymptomatic, most of whom did not even exhibit abnormal erythrocyte sedimentation rate or C-reactive protein, (ii) those who developed mild febrile disease which remitted spontaneously in 24 to 48 hr, and (iii) those whose illness persisted for more than 48 hr but was less severe than the controls. Of the six volunteers in this category, two were ultimately treated with antibiotic.

Results of the challenge studies are summarized in Figs. 1 and 2 in accordance with this classification. Upright figures represent those vaccinees who escaped clinically overt infection. Stooped figures are those with transient illness not requiring therapy, and sitting figures represent vaccinees with modified disease. Those in bed experienced what was judged to be an unmodified illness for which an antibiotic was administered.

Accepting an asymptomatic state and transient illness as evidence of effective immunity,

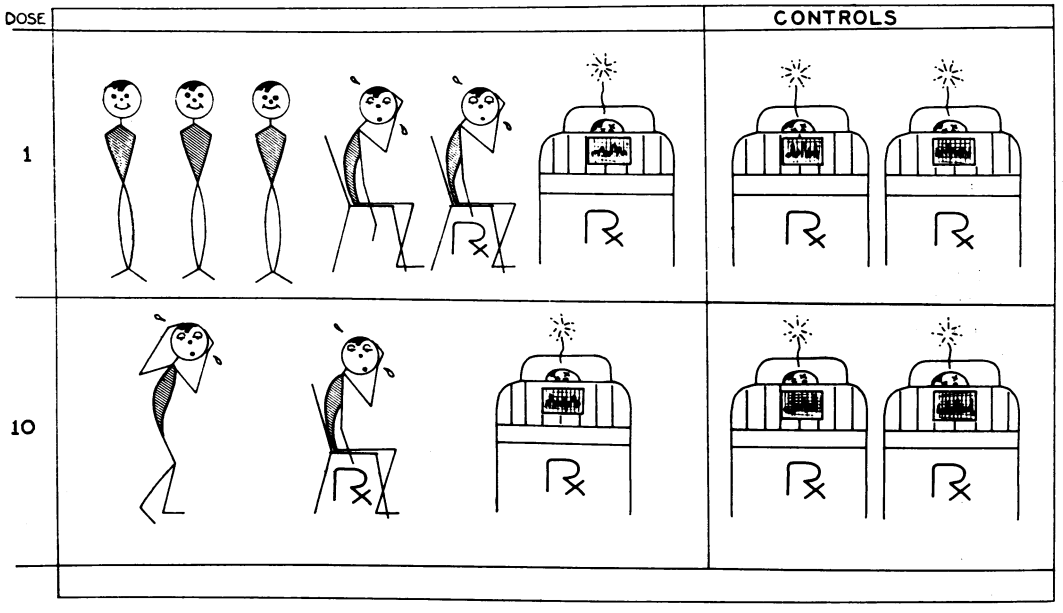

FIG. 1. *Summary of results of challenge in volunteers vaccinated with cell wall vaccine (Larson). Dose is expressed in relative terms; each relative dose being equivalent to 10 human infectious doses.*

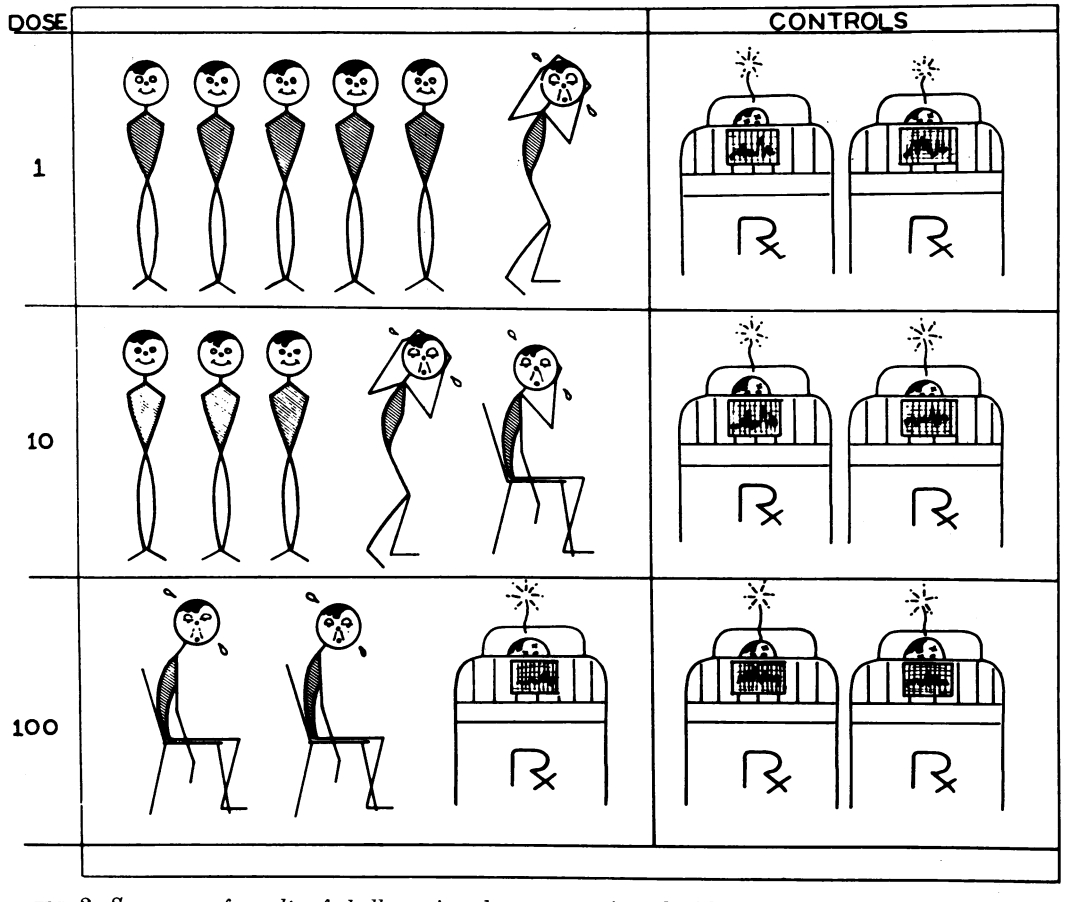

FIG. 2. *Summary of results of challenge in volunteers vaccinated with attenuated tularemia vaccine (Soviet type). Dose is expressed in relative terms; each relative dose being equivalent to 10 human infectious doses.*

it may be concluded that 4 of the 9 Larson vaccinees and 10 of 14 volunteers who received attenuated live vaccine were immune to the type of challenge employed. Three of the four vaccinees who failed to resist challenge were exposed to approximately 1,000 human infectious doses. At the present time, attempts are being made to increase the effectiveness of attenuated vaccine. More recently, Tigertt and Eigelsbach (*unpublished data*) have exposed 24 volunteers to aerosolized vaccine in amounts ranging from 1,500 to 18,000 viable organisms. This exposure was not associated with clinically discernible reaction and the agglutinin response was as good as that observed in percutaneously immunized volunteers.

The difference in mechanism of action of streptomycin on the one hand and broad-spectrum antibiotics on the other has been noted for some time. Several years ago, we were able to demonstrate this difference by treating intracutaneously challenged volunteers with streptomycin and chloramphenicol (7). Streptomycin given initially 1 hr after challenge and for 5 days thereafter apparently eradicated as many as 40,000 *P. tularensis* cells from the inoculation site on the forearm. Chloramphenicol failed to sterilize the inoculation site, as evidenced by the appearance of disease 3 to 4 days after the cessation of therapy. A similar experience was noted in the therapy of aerosol-induced tularemia among control subjects. It appears that either prolonged or intermittent therapy is required with broad-spectrum antibiotics if relapses are to be avoided. Thus, chloramphenicol administered at the rate of 4 g daily for 5 days failed to prevent relapses in 9 out of 11 patients treated for unmodified disease. In some instances, the relapse remitted spontaneously; in others it was promptly controlled with 2 to 3 additional days of chloramphenicol therapy. That this was not always sufficient was evidenced by the occurrence of a second relapse in one of the control subjects.

Of equal interest is the case of a control subject who received 2 g of streptomycin 48 hr after his exposure to 23,000 viable cells of *P. tularensis*. The infection was markedly attenuated by this procedure, which did not interfere with an excellent immune response.

Despite the relative ease of isolating the causative organism from sputum, and gastric and pharyngeal washings, transmission of tularemia from patients to contacts did not occur in either the Overholt et al. (8) series or in the volunteer studies. No attempt was made to isolate the patients. This lack of communicability from man to man is in marked contrast to the facility with which the disease can be induced experimentally or be acquired by breaks in laboratory techniques. The reasons for this difference, whether they be particle size, numbers of organisms, host factors, or others, are beyond the scope of this discussion.

SUMMARY

It may be stated that infection of man with *Pasteurella tularensis* is manifested in various ways. Organisms which gain access to the body through the skin and mucous membranes of the eye or pharynx frequently invade regional lymph nodes and produce a bubonic form of this disease. About 12 to 15% of patients with ulceroglandular tularemia develop pneumonia secondarily. When this microbe is ingested, under certain circumstances, abdominal infection, as described by Soviet workers, follows. The high incidence of pneumonia in typhoidal tularemia suggests that this organism may gain access to the body through the respiratory tract more frequently than has been indicated in the past. Certainly there is no doubt that primary tularemic pneumonia can be induced by an aerosol composed principally of 1-μ particles and that man is regularly infected by as few as 25 organisms when this method is employed.

The absence of lesions in the upper respiratory tract suggests that the pathogenesis of some naturally acquired infections is not dissimilar to the induced disease. If this be the case and typhoidal tularemia results from respiratory exposure, one must admit that the respiratory tract is acting only as the portal of entry in those individuals who do not develop pneumonia. The question of what various sets of circumstances may lead to the syndrome referred to as typhoidal tularemia cannot be answered until more data have been collected from the study of induced infection in man. However, the information available at this time favors the concept that the respiratory tract of man is a sensitive and not infrequent receptor of *P. tularensis*.

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